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ABSTRACT

Purpose: This study aimed to evaluate the antibacterial effect of Apple Vinegar as a root canal irrigant using Endovac irrigation System. **Materials and Methods:** for this study, 60 extracted human teeth were used. After their decapitation, they were instrumented using Universal ProTaper rotary files up to size F4 then separated into 2 set of groups according to the solution used in irrigation; Group A1: irrigated with 5.25 NaOCl Group A2: irrigated with Apple vinegar (30 specimens in each group). We then subdivided each group into 2 subgroups according to the technique used for irrigation; Subgroup A: using conventional irrigation and Subgroup B: using Endovac irrigation system. Samples were sterilized and inoculated with *Enterococcus faecalis* (ATCC29212) for 48 hrs. After irrigation, microbial samples were collected, transferred to nutrient agar and incubated for counting of bacterial colony forming units (CFUs). The significance level was P≤ 0.05. **Results:** There was a statistically significant difference among the tested groups and subgroups in the mean scores of bacterial counts. Concerning the irrigating devices, there was statistically significant difference between conventional irrigation and Endovac system as Endovac showed higher antibacterial effect than conventional irrigation (P ≤ 0.05). Apple vinegar showed statistical significant difference with Endovac system subgroup than conventional irrigation (P ≤ 0.05). **Conclusion** Endovac irrigation system was effective in eradication of E. faecalis from the root canals using either NaOCl or Apple vinegar.

KEYWORDS

Apple vinegar, Antibacterial, Irrigation, Endovac irrigation System.

INTRODUCTION

Microorganisms and their toxic byproducts are of the commonest factors causing pulpal and periapical disease. Thorough disinfection and avoidance of microbial reinfection of root canal system are of the most substantial goals for root canal treatment. Taking into account the

- Paper extracted from master thesis entitled "Evaluation of the Antibacterial Effect of Apple Vinegar as a root canal irrigant using Endovac Irrigation System"

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complex anatomy of root canal systems and also failure of available systems in proper preparation and disinfection of root canal, as 35:80% of canal walls remain underprepared even after its full preparation. Therefore, the combination between chemical and mechanical cleansing of root canals plays a hugely significant rule in root canal disinfection [1].

Proper and ideal irrigation of root canal is known to be considered a substantial element in the success of root canal treatment. Failure is constantly reoccurring in the majority of cases due to the presence of -almost impossible to remove- microorganisms even after proper treatment. Studies stated that almost 45.8% of failed cases are caused by *E. faecalis*, which is a gram positive facultative anaerobe capable of invading dental tubules and also capable of resisting a variety of irrigants and medicaments used in root canal treatment [2].

Sodium hypochlorite (NaOCl) is considered to be the most frequently used irrigant in roots treatment as it is capable of melting down soft tissue, expelling debris out of canals and it also has a vastly wide effect against microorganisms as it has an inhibitory effect on the bacteria's essential proteins, in addition to its antifungal properties. It also has a number of undesired properties such as tissue toxicity, potential allergic reactions, undesirable smell and taste and its inability to remove smear layer. Therefore, several studies are being carried out to find an effective organic substitute [3].

Apple cider vinegar -made from apple cider or apple itself- it has anti-inflammatory and antibacterial characters. Those days, it is vastly used for diabetes and weight loss. Apple cider vinegar is constantly being tested by researchers in dentistry field as a chelating agent, however, few studies were directed towards its antibacterial effects, that's why our current study aims to measure the efficacy of apple cider vinegar as an antibacterial in comparison to the efficacy of NaOCl [4].

Conventional methods of irrigation are vastly used in clinical practice due to its simplicity of application. However, conventional methods have its limitations, as its efficacy depends upon root canal taper, apical preparation, size, design and inserted depth of the needle and irrigant flow. That's why contaminated canals' disinfection using these methods doesn't bring the best results, in addition to the presence of apical vapor lock at the root end which could negatively affect the irrigation's efficacy. Moreover, the positive pressure forming at the tip of the needle may cause extrusion of the irrigant beneath the root to the facial spaces which in turn causes accidents as NaOCl accidents [5].

Apical negative-pressure systems have been also used as it allows delivering the irrigant and sucking it simultaneously which allows delivering the agent along the full length of the root canal. Endovac system being one of the apical negative-pressure systems so, several studies spoke of its efficacy in removal of smear layers and debris from the apical one third of the canal without risking extrusion of the irrigant out of the canal thus reducing post-operative pain. On the other hand, several other studies mentioned its antibacterial effects [6].

Time dependent effect of apical negative pressure (ANP), Vibringe, passive ultrasonic irrigation (PUI), non-activated SAF and conventional irrigation on the reduction of E. faecalis in experimentally infected root canals was evaluated. It came up with a conclusion that irrigation time with conventional needle is extremely affected on the reduction of the bacterial count in the root canal; but there was no significant difference between a time of 2 and 4 minutes of irrigation with the non-activated SAF, Vibringe, Endovac and PUI groups in reducing E. faecalis counts from the root canals [7]. In an in vitro study looked into the effect of time spent in performing the irrigation and the Endovac system effect as an antimicrobial. It came to a conclusion that when increasing the irrigation time, the Endovac system showed improvement in its antibacterial effect [2]; Therefore, the aim of the present study was directed to evaluate the efficacy of apple vinegar as root canal irrigant with Endovac irrigation system in eradication of E. faecalis inside root canal.

MATERIALS AND METHODS

Sample selection and preparation:

For this study, 60 single rooted, single canaled extracted human teeth were chosen with mature apices. Radiographic images of both the mesio-distal and bucco-lingual directions were taken and used for confirming the presence of a single canal. After that, by the use of ultrasonic scaler, the teeth were cleaned out of soft tissue and deposits. The root lengths were standardized to 15 mm by decoronation of the tooth perpendicular to the long axis by a diamond disc.

Universal ProTaper Ni-Ti rotary files were used in a crown-down manner for root canal preparation with a 16:1 reduction hand-piece that was powered by a torque-controlled electric motor; at a rotational speed of 300 rpm and a torque-control of 2.6 N/cm. A set of seven files were used, three shaping files (Sx, S1 and S2) for coronal 2/3 preparation and four finishing files (F1, F2, F3 and F4) for apical 1/3 to provide adequate space for the micro cannula of Endovac irrigating system. During instrumentation, 1 ml of freshly prepared 2.6% sodium hypochlorite (NaOCl) solution was used to irrigate the canal using a 30 gauge Navitip needle. A final rinse was done using 1 mL 17% EDTA, then all the canals were dried with sterile paper points after irrigation. With the use of intermediate restorative material, the root apices were sealed and by the use of nail polish, roots surface were varnished to ensure a closed canal system. All specimens were sterilized using gamma radiation (Cobalt 60 irradiators with dose rate of 1.774 KGY with total dose of 25 KGY).

Biofilm development and canal inoculation:

Sterilized brain heart infusion broth was used as a media for propagation of *Enterococcus faecalis* (ATCC 29212) and incubated at 37°C in anaerobic chamber for 24 hrs. The concentration of bacteria adjusted to 1.5 x 10*8 CFU/ml which is equivalent to #0.5 McFarland turbidity level. All specimens were inoculated by 0.5 mL of the suspension using a micropipette, then the canals were sealed with intermediate restorative material and then, placed individually inside test tubes with 2 ml BHI broth, closed with cotton, inserted inside a rack and placed in the incubator at 37°c for 48 hrs., for allowing bacterial multiplication and proliferation. After incubation, samples were collected from each canal (S1) using sterile paper points and transferred to test tubes containing saline, after 10-fold serial dilutions in sterile saline solution, 0.2 mL were plated onto nutrient agar plates and incubated at 37°C for 48 hrs. Bacterial growth was measured by CFU/ml.

Samples grouping and irrigation procedures:

The sixty samples were divided into two main groups according to irrigation solution (A1) NaOCl & (A2) Apple vinegar with 30 specimens in each group. Each main group was subdivided into two subgroups according to irrigation technique (B1) Conventional irrigation (B2) Endovac with 15 specimens in each subgroup.

In a subgroup B1 for both main groups: For three successive cycles, a 30 gauge Navitip needle was used to deliver 1 ml of the irrigant into the canals by an up and down motion for 30 sec., then the irrigant was left untouched for 60 sec.

In a subgroup B2 for both main groups: Samples were irrigated using Endovac irrigation system, one cycle of macro irrigation in which the syringe tip was used to deliver 1 ml of irrigant. Simultaneously, to allow suction of the irrigant, a macro cannula was then inserted into the canal in an up and down motion for 30 sec., then, the irrigant was left untouched for 60 sec. After macro irrigation, three cycles of micro irrigation were accomplished using a micro cannula. In each cycle, micro cannula was placed at the full working length for 30 sec., to suction the irrigant during its delivering by syringe tip, then, micro cannula was removed and the irrigant was left untouched for 60 s.

Bacterial sampling

After irrigation, the root canals were filled with sterile saline as a transport fluid. The sterile paper points were inserted into the canals and placed until absorbed the transport fluid and was transferred to a test tube containing 1 ml of saline. Each sample was carefully homogenized by vortexing for 30 sec. After incubating the tubes at 37°C in anaerobic chamber for 4 hrs., ten-fold serial dilutions were performed and 100µL of each dilution was plated on nutrient agar and incubated at 37°C for 48 hrs. An automatic colony counter (Flash & Go, IUL, S.A., Barcelona, Spain) used to measure bacterial load as it quantifies colony forming units (CFU)/ ml.

Statistical Analysis

Data were showed as Mean, Standard deviation (SD) values. Data were explored for normality using Kolmogorov Smirnov and Shapiro-Wilk tests and were found not to follow a normal distribution and to be positively skewed so log transformation was made. Mann Whitney U test and Wilcoxon signed rank test were used for interred and intragroup comparisons respectively. The significance level was $P \leq 0.05$ for all tests. Statistical analysis was performed with IBM® SPSS® (SPSS Inc., IBM Corporation, NY, USA) Statistics for windows, version 25.

RESULTS

I. Descriptive statistics:

The highest mean and standard deviation value of bacterial count was found in (A2B1) (Apple vinegar, Conventional irrigation) (3.90±0.50) with the least antibacterial effect followed by (A1B1) (NaOCl, Conventional irrigation) (2.50±0.77) then (A2B2) (Apple Vinegar, Endovac system) (1.66±0.97) while the lowest (mean±SD) value was scored by (A1B2) (NaOCl, Endovac system) (0.67±0.78) with the highest antibacterial effect (Fig. 1).

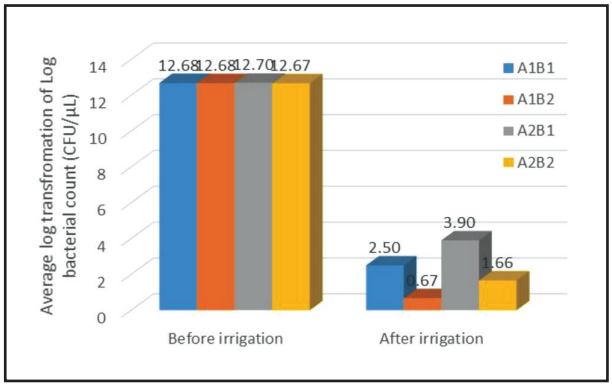

Fig. (1) Bar chart showing average Log bacterial count (CFU/µL) for different groups and subgroups before and after irrigation

II. Effect of Irrigating solution:

After irrigation, there was a statistically significant difference between (NaOCl) and (apple vinegar) groups where ($p \leq 0.05$). The highest mean and standard deviation value was found in (Apple Vinegar (A2)) (2.78±1.37) with less antibacterial effect, while the least mean and standard deviation value was found in (NaOCl (A1)) (1.58±1.20) with higher antibacterial effect.

III. Effect of Irrigation technique:

After irrigation, there was a statistically significant difference between (Conventional irrigation (B1)) and (Endovac (B2)) subgroups where ($p \leq 0.05$). The highest mean and standard deviation value was found in (Conventional irrigation (B1)) (3.20±0.95) with less antibacterial effect, while the least mean and standard deviation value was found in (Endovac (B2)) (1.16±1.00) with higher antibacterial effect.

IV. Effect of Irrigation technique within each Irrigating solution:

Within (NaOCl (A1)) and (Apple Vinegar (A2)) Groups, after irrigation, there was a statistically significant difference between (Conventional irrigation (B1)) and (Endovac (B2)) subgroups where ($p \leq 0.05$).

NaOCl (A1) group: The highest mean and standard deviation value of bacterial count was found in (Conventional irrigation (B1)) subgroup (2.50±0.77) with less antibacterial effect, while the least mean and standard deviation values of bacterial count was found in (Endovac (B2)) subgroup (0.67±0.78) with higher antibacterial effect.

Apple vinegar (A2) group: The highest mean and standard deviation values of bacterial count was found in (Conventional irrigation (B1)) subgroup (3.90±0.50) with less antibacterial effect, while the least mean 865and standard deviation value of bacterial count was found in (Endovac (B2)) subgroup (1.66±0.97) with higher antibacterial effect.

V. Effect of Irrigating solution within each Irrigation technique:

Within (Conventional irrigation (B1)) and (Endovac (B2)) subgroups, after irrigation, there was a statistically significant difference between (NaOCl (A1)) and (Apple vinegar (A2)) groups where ($p \leq 0.05$).

Conventional irrigation (B1) subgroup: The highest mean and standard deviation value of bacterial count was found in (Apple vinegar (A2)) group (3.90±0.50) with less antibacterial effect, while the least mean and standard deviation value was found in (NaOCl (A1)) (1.66±0.78) with higher antibacterial effect.

Endovac (B2) subgroup: the highest mean and standard deviation value of bacterial count was found in (Apple vinegar (A2)) group (1.66±0.78) with less antibacterial effect while the least mean and standard deviation value of bacterial count was found in (NaOCl (A1)) group (0.67±0.78) with higher antibacterial effect (Fig. 2).

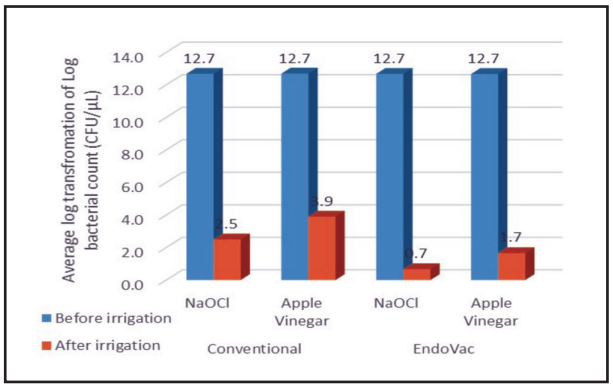

Fig. (2) Bar chart showing average Log bacterial count (CFU/µL) before and after irrigation for different types of Irrigating solutions within each Irrigation technique

DISCUSSION

The existence of microorganisms within root canals is in the form of free-floating cellular entities and dense plaque-like biofilms. The free-floating form is easier to get rid of from the root canal and is more likely to be eradicated by antimicrobial agents; On the other hand, the resistance of bacteria in mature biofilms may exceed 1000-fold more. To ensure the success of root canal treatment, it is substantial to destroy the bacterial biofilms [8]; Therefore, the present study was directed to evaluate the efficacy of apple vinegar as root canal irrigant using Endovac irrigation system in eradication of E. faecalis inside the root canal.

The result of the tested irrigation A1 (NaOCl), and A2 (Apple Vinegar) in present study regardless the irrigating techniques used in the study showed that there was a statistically significant difference between both groups. The highest mean value of bacterial count was found in (Apple vinegar) group with less antibacterial effect while the least mean value of bacterial count was found in (NaOCl) group with higher antibacterial effect. In explanation of the highest antibacterial effect of NaOCl, it was found to be due to the high pH of NaOCl which irreversibly inhibits enzymatic activity, alters cellular metabolism biosynthesis and degrades phospholipids which in turn interferes with the cytoplasmic integrity [9].

Our findings agree with observation of a study which stated that plain apple cider vinegar significantly reduced the E. faecalis numbers after mechanical instrumentation but still less remarkable

than that of sodium hypochlorite when used alone or in combination with apple vinegar [10], But these finding are in contradiction with the results reached by another study in which there were a similarity in antimicrobial effect of both 5% sodium hypochlorite and apple cider vinegar [4]. This difference in the findings might be due to the difference methods. The latter study was done on micro-titer plates not on natural teeth as in our study. In addition, it is in need for further testing to find quantitative analysis rather than its qualitative analysis of the antimicrobial activity of apple cider vinegar.

The use of Endovac irrigation system in the current study showed more antibacterial action compared to the conventional irrigation regardless of irrigation solution, that there was a statistically significant difference between (conventional irrigation) and (Endovac) where the highest mean value of bacterial count was found in (conventional irrigation) with less antibacterial effect while the least mean value of bacterial count was found in (Endovac group) with higher antibacterial effect.

Obtained results in our study were found to agree with other researches which proved Endovac system to be the most effective in debridement and disinfection of the root canal system in comparison to conventional irrigation [11], in another study, the efficacy of Endovac irrigation system to be remarkably better in comparison to the manual irrigation system in primary molars with less extrusion of irrigant amount and better irrigant penetration depth into the dentinal tubule [12]. In another study, after only 48 hrs. of incubation, the efficiency of Endovac irrigation system in removing a thick biofilm of E. faecalis in mesial roots of mandibular molars is established, while after 48 hrs, some of those roots irrigated using conventional irrigation were still found positive [13]. Another study agreed with our study, compared the antimicrobial effectiveness of Endovac system, and conventional irrigation. Out of 16 mandibular molars which was treated with conventional method, negative culture was found in 67 % while 100 % among the Endovac irrigation group [14].

But, our results are in contradiction with an another study that claimed that there is no difference between the two groups; However, "The original Endovac protocol" recommends the use of 5.25% NaOCl [15], Almost all studies investigating the efficacy of Endovac have used concentrations of NaOCl ranging between 2.5 and 6 %. Using 0.5% NaOCl in this study could be the reason for the absence of significant differences in antimicrobial action between Endovac irrigation and conventional irrigation.

CONCLUSION

Based upon the end results of this study it could be concluded that:

1. Out of all the irrigation protocols, none of them totally eradicated E. faecalis, But NaOCl was found to be more effective in eradicating E. faecalis than Apple Vinegar.
2. Endovac irrigation system showed promising action in reducing E. faecalis in root canal system.
3. When combining apple Vinegar with the use of Endovac, it gives more promising results than combining NaOCl with the use of conventional technique.

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